

Application No.: 09/765,555

Docket No.: 278012001420

AMENDMENTS TO THE CLAIMS

1. (currently amended): A method to stably modulate the expression level of a target gene in a plant cell comprising:

a) introducing into a plant cell an expression vector comprising a nucleotide sequence encoding a synthetic zinc finger protein that specifically binds to a target nucleotide sequence, or a complementary strand thereof, within a target gene,

wherein said target nucleotide sequence is of the formula (GNN)₆, wherein N is any one of A, T, C or G ~~comprises 18 nucleotides~~ and wherein said zinc finger protein is a hexadactyl zinc finger protein comprising one individual zinc finger DNA binding site with mutations at one or more of the base-contacting positions; and

b) culturing said plant cell under conditions such that said finger protein is stably expressed and binds to said target nucleotide sequence, whereby the expression of said target gene in said plant cell is stably modulated.

2-3. (canceled)

4. (currently amended): A method to stably modulate the expression level of a target gene in a plant comprising:

a) introducing into a plant cell an expression vector comprising a nucleotide sequence encoding a synthetic zinc finger protein that specifically binds to a target nucleotide sequence, or a complementary strand thereof, within a target gene,

wherein said target nucleotide sequence is of the formula (GNN)₆, wherein N is any one of A, T, C or G ~~comprises 18 nucleotides~~ and wherein said zinc finger protein is a hexadactyl zinc finger protein comprising one individual zinc finger DNA binding site with mutations at one or more of the base-contacting positions;

b) culturing said plant cell under conditions such that said zinc finger protein is stably expressed and binds to said target nucleotide sequence; and

c) growing a plant from the plant cell,

whereby expression of said target gene in said plant is stably modulated.

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5. (original): The method of claim 1, wherein the target nucleotide sequence is endogenous or exogenous to the target gene.

6. (original): The method of claim 1, wherein the target nucleotide sequence is upstream of, downstream of, or within the coding region of the target gene.

7. (original): The method of claim 1, wherein the target nucleotide sequence is DNA, RNA, PNA or a combination thereof.

8. (previously presented): The method of claim 6, wherein the target nucleotide sequence is upstream of the target gene.

9-10. (canceled)

11. (previously presented): The method of claim 1, wherein the target nucleotide sequence comprises 18 consecutive nucleotides with TGA triplets.

12. (canceled)

13. (previously presented): The method of claim 1, wherein the target nucleotide sequence is endogenous to the plant cell but is in a non-naturally-occurring location.

14. (previously presented): The method of claim 1, wherein the plant cell comprises at least two copies of the same or different target nucleotide sequence(s).

15. (original): The method of claim 14, wherein each target nucleotide sequence is located within a different target gene, whereby more than one different target genes are modulated.

16. (previously presented): The method of claim 1, wherein the target gene encodes a protein or a peptide of interest.

17. (canceled)

18. (original): The method of claim 1, wherein the target gene encodes myoinositol 1-phosphate synthase.

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19. (original): The method of claim 1, wherein the target gene encodes a protein and the expression of said encoded protein is modulated.

20. (previously presented): The method of claim 19, wherein the protein whose expression being modulated is heterologous to the plant cell.

21. (original): The method of claim 20, wherein the protein whose expression being modulated is an antibody.

22. (original): The method of claim 19, wherein the expression of the protein is activated.

23-27. (canceled)

28. (previously presented): The method of claim 1, wherein the target gene encodes a protein that confers a desired trait in said plant cell.

29. (previously presented): The method of claim 19, wherein the target gene encodes an enzyme, a transport protein, a nutrient protein, a storage protein, a defense protein or a regulatory protein.

30. (previously presented): The method of claim 29, wherein the target gene encodes an enzyme.

31-35. (canceled)

36. (original): The method of claim 1, wherein the zinc finger protein binds to the complementary strand of the target nucleotide sequence.

37. (original): The method of claim 1, wherein the zinc finger protein specifically binds to an effector domain of the target sequence and whereby the expression of the target gene is modulated by competitive inhibition of said effector domain.

38. (original): The method of claim 37, wherein the zinc finger protein does not comprise an effector domain.

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39. (previously presented): The method of claim 1, wherein the zinc finger protein comprises an effector domain active in the plant cell.

40. (original): The method of claim 1, wherein the zinc finger protein comprises a plurality of finger regions.

41. (original): The method of claim 40, which comprises linker regions among the plurality of finger regions.

42. (previously presented): The method of claim 1, wherein the zinc finger protein comprises at least two 3-finger regions and the linker region between any of said two 3-finger regions is from 2 to 10 amino acid residues in length.

43. (previously presented): The method of claim 42, wherein the linker region between any said two 3-finger regions is about 5 amino acid residues in length.

44. (original): The method of claim 1, wherein the zinc finger protein comprises a framework from a plant zinc finger protein.

45. (canceled)

46. (original): The method of claim 1, wherein the zinc finger protein is selected from the group consisting of ZFPm1, ZFPm2, ZFPm3, ZFPm4 and ZFPAp3.

47. (canceled)

48. (previously presented): The method of claim 1, wherein the plant cell is a monocot plant cell or dicot plant cell.

49. (canceled)

50. (previously presented): The method of claim 1, wherein the plant cell is a protoplast or a spheroplast.

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51. (original): The method of claim 1, wherein the modulation of the gene expression is activation or repression.

52. (previously presented): The method of claim 51, wherein the modulation of the gene expression is at least two fold.

53. (previously presented): The method of claim 51, wherein the modulation is at least five fold repression.

54. (previously presented): The method of claim 51, wherein the modulation is at least two fold activation.

55. (previously presented): The method of claim 4, wherein the modulation of the gene expression changes the phenotype of the plant.

56. (previously presented): The method of claim 4, wherein the plant cell is contained in an *in vitro* culture.

57. (previously presented): The method of claim 4, further comprising growing the plant cell into a plant.

58. (previously presented): The method of claim 1, wherein the expression vector further comprises an inducible promoter.

59. (original): The method of claim 1, wherein the expression of the zinc finger protein is controlled by a tissue-specific promoter and whereby tissue-specific modulation of the target gene expression is obtained.

60. (canceled)

61. (original): The method of claim 1, wherein the zinc finger protein is expressed in a specific organelle.

62. (original): The method of claim 61, wherein the organelle is selected from the group consisting of a mitochondria, a nucleus, a plastid and a vacuole.

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63. (original): The method of claim 62, wherein the plastid is selected from the group consisting of a chloroplast, a leucoplast, an aravloplast and a chromoplast.

64. (previously presented): The method of claim 61, wherein the zinc finger protein is stably integrated in a specific organelle of a plant cell.

65. (previously presented): The method of claim 61, wherein the zinc finger protein is targeted to a specific organelle.

66. (original): The method of claim 65, wherein the zinc finger protein is targeted to plastid via a plastid transit peptide, to chloroplast via a chloroplast transit peptide, to mitochondrial via a mitochondrial target peptide or to nucleus via a nuclear targeting peptide.

67-69. (canceled)

70. (previously presented): A method of stably modulating the level of a compound in a plant cell, which method comprises introducing into a plant cell an expression vector comprising a nucleotide sequence encoding a synthetic zinc finger protein that specifically binds to a target nucleotide sequence, or a complementary strand thereof, within a target gene encoding said compound,

wherein said target nucleotide sequence is of the formula $(GNN)_6$, wherein N is any one of A, T, C or G and wherein said zinc finger protein is a hexadactyl zinc finger protein comprising one individual zinc finger DNA binding site with mutations at one or more of the base-contacting positions; and culturing said plant cell under conditions wherein said zinc finger protein is stably expressed and binds to said target nucleotide sequence, whereby the level of said compound in said plant cell is stably modulated.

71. (original): The method of claim 70, wherein the compound is phytic acid.

72. (original): The method of claim 70, wherein the target gene encodes AP3.

73. (canceled)

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74. (previously presented): An expression vector comprising a nucleotide sequence encoding a synthetic zinc finger protein selected from the group consisting of ZFPm1, ZFPm2, ZFPm3, ZFPm4 and ZFPAp3, said zinc finger protein that specifically binds to a target nucleotide sequence, or a complementary strand thereof, within a target gene.

wherein said target nucleotide sequence comprises 18 consecutive nucleotides and wherein said zinc finger protein is a hexadactyl zinc finger protein comprising one individual zinc finger DNA binding site with mutations at one or more of the base-contacting positions.

75. (canceled)

76. (previously presented): A stably transformed plant cell comprising the expression vector of claim 74, wherein said synthetic zinc finger protein is expressed under the control of a promoter.

77. (previously presented): The stably transformed plant cell of claim 76, wherein the target nucleotide sequence is endogenous or exogenous to the targeted gene.

78. (previously presented): The stably transformed plant cell of claim 76, wherein the target gene is endogenous or exogenous to the plant cell.

79-82. (canceled)

83. (previously presented): The stably transformed plant cell of claim 76, wherein the promoter is an inducible promoter.

84. (canceled)

85. (currently amended): The stably transformed plant cell of claim 76, wherein the zinc finger protein comprises at least six zinc fingers ~~finger sequences~~.

86-87. (canceled)

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88. (previously presented): The stably transformed plant cell of claim 76, which is from a plant selected from the group consisting of a tobacco, tomato, potato, banana, soybean, pepper, wheat, rye, rice, spinach, carrot and corn.

89-90. (canceled)

91. (previously presented): The stably transformed plant cell of claim 78, wherein said zinc finger protein is constitutively expressed.

92. (previously presented): The stably transformed plant cell of claim 78, wherein said zinc finger protein is inducibly expressed.

93. (previously presented): A stably transformed plant tissue, which tissue comprises the stably transformed plant cell of claim 76.

94. (previously presented): A stably transformed plant seed, which seed comprises the stably transformed plant cell of claim 76.

95. (previously presented): The stably transformed plant seed of claim 94, which is from a plant selected from the group consisting of a tobacco, tomato, potato, banana, soybean, pepper, wheat, rye, rice, spinach, carrot and corn seed.

96-97. (canceled)

98. (previously presented): A plant that is regenerated from a plant cell transformed with the expression vector of claim 74.

99. (previously presented): A method to stably modulate the expression level of a target gene in a plant cell, which method comprises culturing the plant cell of claim 76.

100. (previously presented): The method of claim 99 further comprising growing the plant cell into a plant.

101-132. (canceled)

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133. (previously presented): The method of claim 1, wherein the zinc finger protein comprises a framework (or backbone) obtained from a naturally occurring zinc finger protein.

134. (previously presented): The method of claim 1, wherein the zinc finger protein comprises a framework (or backbone) obtained from a zinc finger protein comprising a C2H2 motif.

135. (original): The method of claim 134, wherein the protein or peptide sequence within the β sheet of the C2H2 motif is not changed from its natural sequence.

136. (original): The method of claim 1, wherein the zinc finger protein comprises a framework (or backbone) obtained from a zinc finger protein that is naturally functional in plant cells.

137. (original): The method of claim 136, wherein the framework (or backbone) comprises a motif selected from the group consisting of a C3H zinc finger, a QALGGH motif, a RING-H2 zinc finger motif, a 9 amino acid C2H2 motif, a zinc finger motif of Arabidopsis LSD1 and a zinc finger motif of BBF/D of domain proteins.

138-139. (canceled)